buffer solution, a TAPS (N-Tris-(hydroxymethyl)-methyl-3-aminopropanesulfonic acid) buffer solution, a BES-BisTris buffer solution, a Tris-hydrochloride buffer solution, a 3-morpholinopropanesulfonate (MOPS) buffer solution, and a phosphate buffer solution. The buffer solution are optionally selected depending on the compatibility with the additives before using thereof. In the examples and experiment examples of the present invention, PIPES buffer solution and TAPS buffer solution were respectively used according to a pH condition.

If desired, free cholesterol in the LDL, VLDL and chylomicron may be preliminarily reacted with COD or CDH to convert into cholestenone hydrazone in the presence of hydrazine, to make a non-substrate state for the time when assaying the cholesterol in HDL because the free cholesterol could participate in the reaction upon assaying the cholesterol in HDL to frequently cause errors. The technology for converting the free cholesterol into a non-substrate is well known and reference may be made to Laid-open Japanese Patent Publication Laid-open No. Hei 5-176797, for example.

In the present invention, the above-mentioned three means, i.e., selection of ion strength, selection of enzyme, and selection of surfactant are introduced singly or in optional combinations. More preferably, all of the means are introduced simultaneously. However, it is not always necessary that all means are introduced simultaneously.

In the case where the cholesterol component in the HDL is to be assayed, a first requirement is to control the ion strength to

a sufficiently high level so that a component in the high-density lipoprotein (HDL) having high water solubility can be readily dissolved in the solution. A second requirement is to select lipoprotein lipase (LPL) and/or cholesterol esterase (CE) that preferentially act(s) on the HDL fraction and allow to react therewith. A third requirement is to use a nonionic surfactant that has reaction selectivity for the HDL fraction and having the HLB value of 16 or more and carry out the enzymatic reaction directly or preferentially on a component in the HDL fraction in the reaction solution.

In the case where cholesterol as a component in the lipoprotein by an enzymatic assaying method when CDH is used as the enzyme, β-nicotinamide adenine dinucleotide of the oxide type (NAD), thionicotinamide adenine dinucleotide of the oxide type (t-NAD), β-nicotinamide adenine dinucleotide phosphate of the oxide type (NADP), thionicotinamide adenine dinucleotide phosphate of the oxide type (t-NADP) or the like is used. When COD is used, assay is performed by using a peroxidase (POD) and a known hydrogen peroxide quantitation method in combination. The concentrations of cholic acids as an activator for enzyme and of surfactants that are required for the assay of the total cholesterol may be controlled by optionally selecting the conditions and repeating experiments.

In the case where the cholesterol component in the LDL is to be assayed, first the cholesterol component in the above-mentioned HDL fraction is selectively subject to an enzymatic reaction in a first enzymatic reaction system and then LPL and/or CE that act(s)

on the LDL fraction and a surfactant that positively decomposes LDL are added to thereby detect the reaction product by CDH.

The assay of the enzymatic reaction product is performed by optionally selecting an assay system as shown below from the well-known methods, for quantitating cholesterol that is a compound generated by the enzymatic action of CE, LPL or the like. For example, in the case where a CDH-NAD system is used, the absorbance at 340 nm is measured. In the case where a CDH-t-NAD system is used, the absorbance at 405 nm is measured. In the case where a COD system is used, the absorbance at 500 nm or more (depending on the type of chromogen) is measured. As the photometry, a rate method, a 2-point end method or the like that is already known may be used as desired.

The method for assaying a specific component in the lipoprotein fraction introduced by appropriately selecting means of the present invention singly or in combination do not make turbid in the reaction solution due to complexes or aggregates that will be formed in the reaction liquor. Thus, a precision in assay for the specific object cholesterol in lipoprotein can be increased.

Further, use of the assaying method of the present invention enables to perform assay of the cholesterol in lipoprotein and assay of biochemical test items simultaneously, since simultaneous assay of the biochemical test items will not adversely affect the assay results. Furthermore, the assaying method of the present invention does not need centrifugation and uses a 2-step reagent dispensing operation so that it can be applied to most of the automatic analyzers